Departement für Pferde
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Anton Fürst, Dipl. ECVS

Musculoskeletal Research Unit (MSRU)
Leiterin: Prof. Dr. med. vet. Brigitte von Rechenberg, Dipl. ECVS

Arbeit unter wissenschaftlicher Betreuung von
Dr. med. vet. Peter W. Kronen, Dipl.ECVAA

Clinical evaluation of intranasal medetomidine-ketamine and medetomidine-S(+)-ketamine for induction of anaesthesia in rabbits in two centres with two different application techniques

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Linda Weiland

Tierärztin

aus Luxemburg-Stadt, Luxemburg

genehmigt auf Antrag von

Prof. Dr. med. vet. Brigitte von Rechenberg, Referentin

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Summary

Vetsuisse-Fakultät Universität Zürich 2016

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Clinical evaluation of intranasal medetomidine-ketamine and medetomidine-S(+)-ketamine for induction of anaesthesia in rabbits in two centres with two different application techniques

The aim was to study the efficacy and side effects of induction with medetomidine-ketamine and medetomidine-S(+)-ketamine by intranasal instillation (IN) in rabbits and to evaluate both protocols during subsequent isoflurane anaesthesia. The prospective, blinded, randomized experimental study was performed in two centres with 83 healthy New Zealand White rabbits. Medetomidine (0.2 mg/kg) with 10 mg/kg ketamine (MK) or 5 mg/kg-S(+)-ketamine (MS) was administered IN to each rabbit. 39 animals were in group MK and 44 in group MS. In Centre 1 rabbits (42) were held in sternal and in Centre 2 (41) rabbits were in dorsal recumbency during IN instillation. If a rabbit swallowed during endotracheal intubation, half of the initial IN dose was repeated and intubation re-attempted after 5 minutes. Heart rate, blood pressure, end-tidal carbon dioxide concentration, and blood gases were recorded during anaesthesia. Data were statistically analysed.

Two rabbits died after instillation of the drug. Eight (MK) and 11 (MS) rabbits were insufficiently anaesthetized and received a second IN dose. One rabbit in MK and three rabbits in MS required an isoflurane mask induction. There were no significant differences between treatments for induction, intraoperative and recovery data.

This study indicated that MK and MS were effective shortly after IN delivery but in dorsal recumbency IN administration led to two fatalities (MS). Factors leading to death have not been fully elucidated.

Keywords

Intranasal, rabbit, medetomidine, S(+)-ketamine, ketamine

Zusammenfassung

Vetsuisse-Fakultät Universität Zürich 2016

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Klinische Bewertung intranasaler Verabreichung von Medetomidin-Ketamin und Medetomidin-S(+) Ketamin zur Anästhesieeinleitung beim Kaninchen aus zwei Zentren mit zwei verschiedenen Techniken

Die Wirksamkeit und Nebeneffekte von Medetomidin-Ketamin und Medetomidin-S(+)-Ketamin wurden zur Anästhesieeinleitung nach intranasaler Applikation (IN) beim Kaninchen untersucht. Beide Protokolle wurden während der anschließenden Erhaltung mit Isofluran evaluiert. Die prospektive, verblendete, randomisierte Experimentalstudie fand an 83 gesunden, weiblichen Weissen Neuseeländer Kaninchen in 2 Zentren statt.
Jedem Tier wurde Medetomidin (0.2 mg/kg) mit 10 mg/kg Ketamin (MK) oder 5 mg/kg S(+)Ketamin (MS) IN verabreicht. 39 Tiere wurden der Gruppe MK und 44 der Gruppe MS zugeordnet. Im Zentrum 1 wurden die Tiere in Brustlage (42) und im Zentrum 2 in Rückenlage (41) positioniert. Wenn ein Tier während der Intubation schluckte, wurde die 1/2 der initialen IN Dosis erneut verabreicht und eine Intubation nach 5 Minuten wieder versucht. Herzfrequenz, Blutdruck, end-tidale Kohlendioxidkonzentration und die Blutgaswerte wurden während der Narkose notiert. Alle Daten wurden statistisch ausgewertet.
Zwei Tiere starben nach der Verabreichung der Medikamente. 8 (MK) und 11 (MS) Tiere benötigten eine zweite IN Dosis. Ein Kaninchen in MK und 3 in MS benötigten eine Isofluran-Maskeneinleitung. Es gab keine signifikanten Unterschiede hinsichtlich der Einleitung, intraoperativen Daten und der Aufwachphase.
Diese Studie zeigt, dass MK und MS kurz nach Verabreichen der IN Dosis wirksam waren. Jedoch verstarben in Rückenlage zwei Tiere (MS). Die genaue Todesursache konnte nicht ermittelt werden.

Stichworte: Intranasal, Kaninchen, Medetomidin, S(+) Ketamin, Ketamin

Titel Manuskript

Clinical evaluation of intranasal medetomidine-ketamine and medetomidine-S(+)-ketamine for induction of anaesthesia in rabbits in two centres with two different administration techniques

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Keywords: Intranasal, rabbit, medetomine, S(+)-ketamine, ketamine, intubation

Running Title: Intranasal anaesthesia in rabbits

Abstract

Objective

The aim was to compare efficacy and side effects of induction with medetomidine–ketamine or medetomidine–S(+)-ketamine by intranasal (IN) instillation in rabbits and to evaluate both protocols during subsequent isoflurane anaesthesia.

Study design

Prospective, blinded, randomized experimental study in two centres.

Animals Eighty-three healthy New Zealand White rabbits undergoing tibial or ulnar osteotomy.

Methods

Medetomidine (0.2 mg kg–1) with 10 mg kg–1 ketamine (MK) or 5 mg kg–1 S(+)-ketamine (MS) was administered IN to each rabbit in a randomized fashion. In Centre 1 (n = 42) rabbits were held in sternal recumbency, and in Centre 2 (n = 41) in dorsal recumbency, during drug instillation. Adverse reactions were recorded. If a rabbit swallowed during endotracheal intubation, half of the initial IN dose was repeated and intubation was re-attempted after 5 minutes. Anaesthesia was maintained with isoflurane. Heart rate, blood pressure, end-tidal carbon dioxide concentration and blood gases were recorded. Data were analysed using Student's t-test, Mann–Whitney test and Fisher's exact test.

Results

In all, 39 animals were assigned to the MK group and 44 to the MS group. Two rabbits in the MS group held in dorsal recumbency died after instillation of the drug. Eight (MK) and 11 rabbits (MS) were insufficiently anaesthetized and received a second IN dose. One rabbit in MK and three in MS required an isoflurane mask induction after the second IN dose. There were no significant differences between treatments for induction, intraoperative data, blood gas values and recovery data.

Conclusion and clinical relevance

This study indicated that medetomidine–ketamine and medetomidine–S(+)-ketamine were effective shortly after IN delivery, but in dorsal recumbency IN administration of S(+)-ketamine led to two fatalities. Nasal haemorrhage was noted in both cases; however, the factors leading to death have not been fully elucidated.

Keywords

endotracheal intubation, intranasal, ketamine, medetomidine, rabbit, S(+)-ketamine.

Introduction

Rabbits are common companion animals and are also widely used in an experimental setting. However, safe anaesthesia of adequate depth and duration remains challenging in rabbits. The anaesthetic-related fatality rate has been found to be significantly higher in rabbits (1.39%) than in dogs (0.17%) and cats (0.24%) (Brodbelt et al. 2008). Development of safe anaesthetic protocols and techniques for rabbits is therefore a priority.

A number of injectable anaesthetic techniques for rabbits have been described. Combinations of medetomidine and ketamine alone (Orr et al. 2005), or together with buprenorphine (Murphy et al. 2010) or butorphanol (Hedenqvist et al. 2002) have been used successfully in rabbits. The drugs were administered by intramuscular (IM), intravenous (IV) or subcutaneous (SC) injection.

In recent years, interest in the development of alternative drug delivery routes, such as the intranasal route (IN), has been growing (Pereira et al. 2011; Grassin-Delyle et al. 2012). IN delivery is a simple and convenient method of application and reduces the likelihood of first-pass metabolism compared with oral administration. The respiratory mucosa, which constitutes the main site for drug deposition and absorption, is highly vascularized and the time to effect can be as rapid as IV administration (Weber et al. 2004). In addition, the olfactory epithelium enables direct access to the central nervous system by bypassing the blood–brain barrier. Absorption can be limited, though, by mucociliary clearance of the nasal cavity (Pires et al. 2009), swallowing, inhalation of the drug, the volume of fluid administered and the animal's position during drug instillation.

Intranasal administration of sedative drugs has been documented for therapeutic and experimental purposes in a number of different species, including rabbits (Gizurarson 1990; Lindhardt et al. 2002; Kaur & Kim 2008), and a number of different drugs, including α2-adrenoceptor agonists, ketamine and S(+)-ketamine (Robertson & Eberhart 1994; Vesal & Eskandari 2006; Vesal & Zare 2006). The S(+)- isomer of ketamine has been used in veterinary medicine and evaluated in ponies (Larenza et al. 2007), dogs (Duque et al. 2008), rabbits (Cruz et al. 2010; Sponheimer 2010) and hares (Gerritsmann et al. 2012). Reports in humans indicate that S(+)-ketamine isomer may produce shorter recoveries with fewer psychological side effects compared with racemic ketamine (Pai & Heining 2007).

Therefore, this study aimed to evaluate whether IN administration of medetomidine in combination with ketamine or S(+)-ketamine produces acceptable loss of consciousness and the swallowing reflex to allow for endotracheal intubation in healthy New Zealand

White (NZW) rabbits. The drugs were administered in two different body positions. Complications and cardiopulmonary data during subsequent isoflurane anaesthesia were recorded.

Materials and methods

All procedures were conducted in accordance with the local animal use and welfare laws (ethics committee permission number: BE 59/05 and GR 1/05). In this randomized, prospective, blinded study conducted in two centres, 83 young adult NZW rabbits (Charles River Laboratories, France) underwent experimental tibial critical size defect surgery (Centre 1) or ulnar osteotomy (Centre 2). The number of animals used was determined by the respective orthopaedic surgical study.

They were group-housed in floor pens on dust-free wooden shavings in a room with a 12:12 hour light:dark cycle. Room temperature was maintained at 18 ± 2 °C, and relative humidity at 40–60%. They were fed a commercial pelleted diet (Kliba-Nafag, Switzerland) and were offered water ad libitum, supplemental hay and fresh vegetables daily. Food and water were not withdrawn before surgery.

All rabbits were considered healthy based on history and a preanaesthetic clinical examination. The lungs and hearts of the rabbits were auscultated and a baseline heart rate (HR) was recorded. Medetomidine 0.2 mg kg–1 (Domitor, 1 mg mL–1; Pfizer, Orion Corporation, Finland) and 10 mg kg–1 ketamine (group MK; Ketasol-100 ad us.vet., 100 mg mL–1; Dr E. Graeub AG, Switzerland) or 5 mg kg–1 S(+)-ketamine (group MS; Keta-S ad us.vet., 60 mg mL–1; Dr E. Graeub AG) diluted in 0.9% saline to identical final volumes (0.3 mL kg–1) were randomly assigned to each rabbit (http://www.randomizer.org). The drug mixtures were pre-prepared in coded syringes by an assistant to ensure that the attending anaesthetist was unaware of the treatment group.

Half of the mixture was administered via a catheter-tipped syringe (Surflo 22 gauge × 1"; Terumo, NJ, USA) into each nostril over 30 seconds. In Centre 1, rabbits were restrained in the sternal position with the head and neck gently dorsoflexed during the procedure by an assistant. They were kept immobile for approximately 30 seconds after nasal delivery by the anaesthetist. In Centre 2, the drug mixture was administered by the anaesthetist who also held the rabbits in dorsal recumbency with their neck scruffed. Reactions during drug administration (e.g. sneezing, swallowing, head shaking, dyspnoea and epistaxis) were recorded.

Rabbits were returned to their boxes and, 5 minutes after IN application, a numeric rating score for sedation was allocated, as follows: 1, deep (loss of righting reflex or lateral recumbency); 2, moderate (lateral recumbency and mild struggling when handled); 3, poor (sternal recumbency and immediate marked struggling when handled); and 4, absent sedation. The pedal withdrawal reflex was also tested at this time point. If after 5 minutes the sedation score was 1, lidocaine 1–2 mg kg–1 (Lidocain 2%, 20 mg mL–1; Streuli, Switzerland) was applied topically onto the larynx for desensitization. In Centre 2, the rabbits were preoxygenated using a face mask. Endotracheal intubation with a cuffed 3.5 or 4.0 mm internal diameter endotracheal tube (Blue Line Tracheal Tube; Covidien, Switzerland) was attempted using an acoustic (nonvisual) technique with a modified stethoscope, as described previously (Sponheimer 2010). Rabbits with a sedation score ≥ 2 were re-evaluated in the cage after a further 5 minutes. If sedation remained insufficient or if a rabbit demonstrated a swallowing reflex during endotracheal intubation, half of the initial dose of the drug mixture was repeated IN, and intubation was re-attempted 5 minutes later. If the endotracheal tube could not be placed after the second dose, a mask induction was performed with isoflurane in oxygen to allow endotracheal intubation. The anaesthetist scored the ease of intubation with a subjective numeric rating score, as follows: 1, good (intubation with no swallowing and head movement); 2, moderate (one swallowing attempt, no head movement) ; and 3, poor (more than one swallow and head movement).

Rabbits received subcutaneous enrofloxacin (10 mg kg–1, Baytril 2.5%; Bayer, Switzerland) and the eyes were lubricated (Lacryvisc; Alcon Pharmaceuticals Ltd, Switzerland). Two over-the-needle catheters (Surflo 22 gauge × 1"; Terumo, NJ, USA) were placed in the left auricular vein and in the right auricular artery after endotracheal intubation. The arterial catheter was connected to a pressure transducer (Codan Medical AG, Switzerland), which was aligned with the glenohumeral joint and zeroed. An arterial blood sample (0.5 mL) was then withdrawn via the arterial catheter (at time T0) and analysed for blood-gas variables (ABL800 Flex Blood Gas Analyzer; Radiometer, Denmark); this was repeated after 30 minutes (T30). Anaesthesia was maintained with isoflurane (Attane Isoflurane ad us.vet.; Provet, Switzerland) in 1.5 L minute–1 oxygen via a non-rebreathing (Bain) anaesthetic breathing system (Intersurgical, Germany). The osteotomies were performed by one single experienced surgeon per centre. The concentration of delivered isoflurane was altered based on the assessment of HR and spontaneous movements. HR, invasive arterial blood pressure and end-tidal carbon dioxide tension (PE´CO2) were measured continuously with a multiparameter monitor (Centre 1: AS/3 compact; Datex-

Ohmeda, Finland; Centre 2: CMS24 Omni Care M1204A, HP, Switzerland) and recorded every 5 minutes. All animals were positioned in lateral recumbency and were warmed using a forced-air warming device (Bair Hugger; Augustine Medical, MN, USA). An infusion of 10 mL kg–1 hour–1 of Ringer's lactate (Ringer-Lactat-Lösung Fresenius; Fresenius Kabi AG, Switzerland) was administered IV. If blood pressure decreased by 20% or more, the rabbits received an IV infusion of dobutamine 2–4 µg kg–1 minute–1 (Dobutrex, 5mg mL–1; Medika AG, Switzerland) to effect.

Buprenorphine [0.02 mg kg–1 (Centre 1) or 0.05 mg kg–1 (Centre 2); Temgesic; Essex Chemie AG, Switzerland) was injected IV 30 minutes before the end of anaesthesia. At the end of the procedure, rabbits were placed in lateral recumbency, actively warmed and observed. Rectal temperature was measured using a digital thermometer (VT 1831; Microlife, Switzerland), and carprofen [2 mg kg–1 (Centre 1) or 4 mg kg–1 (Centre 2); Rimadyl, 50 mg mL–1; Pfizer, Switzerland] was injected SC. Buprenorphine was administered SC every 8 hours for the following 3 days.

Statistical analysis

Data management and statistical analysis were performed using Excel for Macintosh (Microsoft Office 2011; Microsoft Corp., WA, USA) and Prism version 4.0b (GraphPad Software, Inc., CA, USA). All variables were tested for normal distribution using the Kolmogorov–Smirnov test.

Duration of anaesthesia was taken to be the time from endotracheal intubation to extubation. Normally distributed data were analysed using a Student's t-test, with results presented as mean ± standard deviation. Non-normally distributed data were compared using a Mann–Whitney test, with the results presented as median (range). Fisher's exact test was used to compare categorical outcomes. A value of $p < 0.05$ was considered statistically significant.

Results

In all, 83 rabbits were included in the study, 39 of which received medetomidine–ketamine and 44 medetomidine–S(+)-ketamine. In Centre 2 (n = 41), after drug administration in dorsal recumbency, two of the 20 rabbits receiving S(+)-ketamine exhibited severe haemorrhage from both nostrils. In one rabbit, epistaxis occurred after the initial dose of MS, and in the second rabbit epistaxis only occurred after a second dose of

MS. Both rabbits had become recumbent when epistaxis was first observed and were immediately removed from their cages. Apnoea and presence of epistaxis delayed endotracheal intubation, but both animals were successfully intubated and this was confirmed by capnography. After decrease of PE´CO2 to zero and loss of a palpable pulse, cardiopulmonary resuscitation was attempted, but a return of spontaneous circulation was never achieved. Large quantities of intrapulmonary blood were found on post-mortem examination of both animals. No histology was performed. All 42 rabbits in Centre 1 completed the study without complications.

Demographic data and group allocation can be found in Table 1. The age ($p = 1.00$) and weight ($p = 0.87$) were similar between the MK and MS groups; however, older and heavier animals were included in Centre 1 (both $p < 0.0001$).

Induction data

The volumes of drugs administered in the first dose were 1.2 ± 0.2 (0.9–1.7) mL for the MK group and 1.2 ± 0.2 (0.9–1.6) mL for the MS group. In Centre 1, two rabbits in the MK group sneezed immediately after the first IN application, one swallowed and two struggled or shook their heads. In the MS group, three animals immediately swallowed, one sneezed and two struggled or shook their heads during the first IN administration. In Centre 2 all animals showed signs of dyspnoea after IN administration.

At 5 minutes after IN administration, pedal withdrawal reflex was positive in four out of 39 animals (10%) in the MK group, and four out of 44 animals (9%) in the MS group ($p = 1.00$). The requirement for a second drug administration was similar in both groups: eight out of 39 (21%) rabbits in the MK group and 11 out of 44 (25%) rabbits in the MS group ($p = 0.79$). One animal in the MK group and three in the MS group required masked isoflurane for completion of anaesthetic induction ($p = 0.37$). Median (range) times from initial IN application to positioning for successful endotracheal intubation were similar between groups, with 8 (5–32) minutes in the MK group versus 10 (5–40) minutes in the MS group ($p = 0.15$). No significant differences were found between the MK and MS groups for sedation scores [1 (1–4), $p = 0.28$] and intubation scores [1 (1–4), $p = 0.59$].

Intraoperative data

The duration of anaesthesia was not significantly different between the MK and MS groups [70 (25–133) versus 66 (35–120) minutes; $p = 0.44$], but it was significantly shorter in Centre 1, with a median of 60 (25–120) minutes versus one of 78 (50–133) minutes ($p < 0.0001$). HR, systolic blood pressure, PE´CO2 (Table 2) and blood-gas results were similar in both treatment groups (Table 3).

Recovery data

Mean body temperature at extubation was within normal limits. Extubation time was similar between groups [6 (1–20) minutes in the MK group versus 5 (1–24) minutes in the MS group; $p = 0.63$], but a statistically significant shorter extubation time was found in Centre 1 ($p < 0.0001$) and this is probably related to shorter anaesthesia times and the lower buprenorphine dose in Centre 1. In all animals that were recovered from anaesthesia, recovery was smooth and uneventful.

Discussion

This study aimed to describe the clinical evaluation of IN medetomidine in combination with ketamine or S(+)-ketamine in rabbits. The selected doses of ketamine and S(+)-ketamine were lower than most published studies using IM or SC administration and resulted in 71% of all animals being sufficiently anaesthetized for endotracheal intubation. In the second centre, the IN administration performed in dorsal recumbency led to two fatalities after medetomidine and S(+)-ketamine. The overall fatality rate of 2.4% in the current study is higher than the reported rate of 1.39% (Brodbelt et al. 2008), even though healthy research animals were anaesthetized.

The drugs were administered IN with the aid of a catheter, the tip being inserted into the ventral meatus of the nostril. The position of the animal during administration and the exact method of delivery (speed, site of catheter within nasal cavity, etc.) can influence drug deposition and absorption after IN administration. A pharmacokinetic study investigating the differences between supine and sitting positions during IN administration of drugs in rabbits demonstrated that the amount absorbed was greatly affected by the application technique (Gizurarson et al. 2006). Rabbits in Centre 1 were in a sternal position but with the head dorsiflexed. In Centre 2, the supine position may have led to a different distribution and a very rapid onset of action. Overdosage of medetomidine or ketamine is a possible explanation for the death of two animals, although this would not be consistent with the intrapulmonary blood.

Another possible reason could be an increased difficulty of IN application when the rabbit is held in dorsal recumbency and a catheter is inserted into the nose by the same person. This technique might have led to increased damage to the highly vascularised and sensitive areas in the nose. Such damage could have caused significant bleeding, possibly explaining the large amount of blood found in the lungs of both dead rabbits. Lung bleeding

might also have been caused by direct contact of the drug or its preservative with the lung. The IN use of S(+)-ketamine without preservatives is described in humans (Johansson et al. 2013), as opposed to the current study, where S(+)-ketamine with preservative (sodium-methyl-paraben E 219) was used. Severe pulmonary hypertension with disruption of pulmonary vessels induced by medetomidine is another possible mechanism, which has been previously described in sheep after IV administration of xylazine (Celly et al. 1999). However, pulmonary oedema but not gross pulmonary bleeding was observed in affected sheep and such side effects were neither seen in the current study nor previously described in rabbits.

The other adverse reactions during IN application were sneezing, swallowing and head shaking. IN dexmedetomidine, ketamine and S(+)-ketamine have been used previously in children and adults, and were described to not cause any discomfort or sensation of burning (Diaz 1997; Yuen et al. 2007; Huge et al. 2010; Cheung et al. 2011). In a follow-up study with 16 rabbits in the sternal position in Centre 1, drugs were instilled into the nose without insertion of a catheter, and no sneezing, head shaking or struggling was observed. Interestingly, all four animals that showed head shaking were observed to have ocular discharge or mild conjunctivitis at the preoperative clinical exam. These reactions could be due to congestion of the nasal mucosa or an increased amount of mucus, leading to a diminished air passage.

In contrast to another study, in which 50% of the animals given IN xylazine-ketamine showed increased salivation, we did not see this side effect in our study (Robertson & Eberhart 1994).

The rabbits in Centre 2 showed signs of dyspnoea after IN drug delivery. The volume applied to each nostril was large (about 0.6 mL per nostril). Most previous studies in rabbits have used a total volume of 0.05–0.15 mL per nostril (Bechgaard et al. 1997; Lindhardt et al. 2002; Gizurarson et al. 2006). The total volume of the nasal cavities of an adult NZW rabbit is reported to be 6 mL and the nasal surface area is 61 cm2 (Gizurarson 1990). Robertson & Eberhart (1994) demonstrated, by giving 0.2 mL of contrast dye per nostril to Flemish Giants and NZW rabbits, that most of the fluid reached the vomeronasal organ and some liquid was swallowed. The consequence of excessive fluid is pharyngeal deposition, swallowing, gut absorption and possible pre-systemic metabolism. Aspiration of the drug is another possible consequence. The permissible volume of solution for IN administration is relatively low, and therefore drugs with low aqueous solubility and/or requiring high doses may present a problem (Pires et al. 2009). Unfortunately, the current commercial

formulations of medetomidine, ketamine and S(+)-ketamine are such that large volumes are required. In the human literature, aerosolized drugs are used for IN application (Johansson et al., 2013), potentially enhancing the distribution onto the nasal mucosa. However, most aerosols usually deliver 0.1 mL per spray, so that repetition is required for administration of the total dose.

Most animals (71%) in this study were adequately anaesthetized to permit endotracheal intubation after IN drug application. The precise site of drug absorption was not determined in this study, although the rapid onset of sedation indicated that a significant portion of the drug mixture was probably absorbed via the nasal mucosal route. The drug doses used in this study were lower than those previously published, in which doses of medetomidine were 0.2–0.5 mg kg–1 with ketamine 15–35 mg kg–1 (Hedenqvist et al. 2002; Orr et al. 2005; Murphy et al. 2010). Reports of S(+)-ketamine use in rabbits exist only for co-induction with propofol after sedation with acepromazine and butorphanol (Cruz et al. 2010) and at a dosage of 7.5 mg kg–1 for SC administration with medetomidine 0.2 mg kg–1 (Sponheimer 2010) . As all these studies used different end points, a direct comparison of dosages and time to onset among the IM, SC and IN routes of administration would only be possible by studies including all three routes of administration.

Time to lateral recumbency was, in many rabbits in our study, less than 5 minutes after IN application. In contrast, rabbits receiving IM and SC medetomidine–ketamine (0.25 mg kg–1 and 15 mg kg–1, respectively) combinations demonstrated a much longer mean onset of loss of pedal withdrawal reflex: 6.6 minutes in the IM group and 12.8 minutes in the SC group (Orr et al. 2005). Such slow induction of anaesthesia is potentially harmful in rabbits, inducing stress in this easily agitated species, and so a rapid induction and endotracheal intubation after IN administration are desirable.

Based on various studies in humans and animals, S(+)-ketamine exhibits two-fold greater analgesic and hypnotic properties than the R(-) isomer, and a 50% reduction of dose of the racemic mixture is usually considered adequate to achieve comparable results (White et al. 1985; Duque et al. 2008). Our study supports this finding, although there was a trend to longer intubation times with S(+)-ketamine ($p = 0.15$), suggesting that the enantiomer has a potency slightly lower than 50% of the racemate. A potency relationship calculation cannot be performed with our data; however, Sponheimer (2010) found a relative potency of 1:1.6 of ketamine and S(+)-ketamine in rabbits. Currently, S(+)ketamine is commercially formulated as 60 mg mL–1 to achieve the same potency per mL as with ketamine 100 mg mL–1.

No intraoperative statistical differences were found in HR, blood pressure, PE´CO2 and blood gases between the MK and MS groups in the first 15 minutes of isoflurane anaesthesia. These results should not be over-interpreted as neither the time from first application nor the total dose of drugs before recorded cardiopulmonary measurements were standardized in the current study, leading to variable plasma levels of MK and MS during cardiopulmonary data retrieval. However, our results are similar to comparative studies between ketamine and S(+)-ketamine in goats (Jud et al. 2010) and hares (Gerritsmann et al. 2012) but not in ponies (Larenza et al. 2007).

In the present study, recovery of the 81 surviving rabbits was smooth and uneventful. Although S(+)-ketamine is reported to have a faster plasma clearance than the racemic mixture (Duque et al. 2008; Larenza et al. 2008), no significant differences between MK and MS regarding extubation times were noticed. IN pharmacokinetic data in rabbits are not published, but it is unlikely that after an anaesthesia time of more than 1 hour, the choice of ketamine versus S(+)-ketamine had an impact on recovery time.

Some limitations are inherent to this study. The longer anaesthesia time combined with a 2.5 times higher buprenorphine dose could explain the significant difference in duration of recovery between centres. It is also debatable whether the animals used in the current study are to be considered healthy when showing eye discharge or mild conjunctivitis. However, no signs of ill health were detected upon clinical examination of the animals that died after induction of anaesthesia.

As a further limitation, time to onset could not be determined precisely in the current study as the first time point was set at 5 minutes after IN application. Rabbits from all four groups (MS and MK at both centres 1 and 2) showed drug effects before 5 minutes and future studies should include earlier time points. The lidocaine applied topically on the larynx might have been absorbed systemically and this could have exerted an additional sedative effect in the rabbits. As the rabbits were of a similar weight in the MK and MS groups, with the same dose of lidocaine, no effect on the difference of the two tested protocols was expected. The possible inter-rater difference in sedation and intubation scoring between the centres is a further limitation of the current study. The definition of numeric rating scores potentially reduced variation between researchers. Inter-centre differences in age, weight, preoxygenation practices, buprenorphine dosage, anaesthesia time and recovery time were present but evenly distributed between the MK and MS groups and did not lead to any statistically significant differences between the treatments. No sample size was calculated for the current study, because the number of animals was

determined by the respective orthopaedic study. Further studies would be required to assess pharmacokinetic profiles and brain absorption of MK and MS.

Conclusion

This study indicates that 0.2 mg kg–1 medetomidine with either 10 mg kg–1 ketamine or 5 mg kg–1 S(+)-ketamine allowed for endotracheal intubation shortly after IN delivery, but in dorsal recumbency, IN administration of S(+)-ketamine led to two fatalities. The potential causes of death cannot be fully determined from the data in this study, but include the drugs themselves, preservatives, the volume of fluid, a catheter-induced injury of the nasal mucosa and the position of the animal during application

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Table 1

Demographic data and group allocation of 83 rabbits receiving either medetomidine–ketamine (MK) or medetomidine S(+)–ketamine (MS) intranasally. Body positions during drug administration were different according to centre, with sternal positioning (S) in Centre 1 and dorsal recumbency (D) in Centre 2

Group	**MK**		**MS**	
	Centre 1	**Centre 2**	**Centre 1**	**Centre 2**
Number of animals (*n*)	18	21	24	20
Position	S	D	S	D
Death rate (*n*)	0	0	0	2
Sex				
Male (*n*)	0	18	0	20
Female (*n*)	18	3	24	0
Weight (kg)	4.6 ± 0.5 (3.8–5.7)	3.5 ± 0.3 (3–4.3)	4.6 ± 0.5(3.7–5.5)	3.3 ± 0.2 (2.9–3.7)
Age (months)	12	4	12	4

Results are presented as mean ± standard deviation or (range).

Table 2

Heart rate (HR), systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressures and end-tidal carbon dioxide ($P_{E'}CO_2$) in rabbits receiving medetomidine–ketamine (MK) or medetomidine–S(+)-ketamine (MS) intranasally. Values from the first 15 minutes of anaesthesia maintained with isoflurane in 100% O_2 are presented as mean ± standard deviation. No differences between the groups were noted

	Time (minutes)		
Variable	**5**	**10**	**15**
HR			
MK	168 ± 26	178 ± 35	178 ± 34
MS	160 ± 33	169 ± 40	170 ± 38
p	0.30	0.29	0.34
SAP			
MK	78 ± 17	76 ± 12	76 ± 9
MS	73 ± 13	76 ± 14	74 ± 12
p	0.25	0.97	0.56
MAP			
MK	62 ± 14	62 ± 8	61 ± 10
MS	61 ± 12	64 ± 12	63 ± 12
p	0.76	0.52	0.47
DAP			
MK	51 ± 12	51 ± 7	51 ± 10
MS	49 ± 11	54 ± 12	52 ± 13
p	0.57	0.35	0.66
$P_{E'}CO_2$			
MK	45 ± 11	44 ± 12	45 ± 11

MS	44 ± 8	46 ± 8	44 ± 10
p	0.72	0.65	0.76

Table 3

Blood gas values immediately after placement of the arterial catheter (T0) and 30 minutes thereafter (T30) in rabbits receiving intranasal medetomidine–ketamine (MK) and medetomidine–S(+)-ketamine (MS). Anaesthesia was maintained with isoflurane in 100% O2. Data are presented as mean ± standard deviation. No differences between the groups were noted

Variable	**MK**	**MS**	***p*-value**
pH (T0)	7.30 ± 0.08	7.29 ± 0.07	0.66
pH (T30)	7.31 ± 0.08	7.3 ± 0.05	0.44
$PaCO_2$ (T0)			
(mmHg)	49.0 ± 5.9	49.8 ± 8.3	0.68
(kPa)	6.5 ± 0.8	6.5 ± 0.8	
$PaCO_2$ (T30)			
(mmHg)	53.8 ± 5.6	54.0 ± 8.4	0.94
(kPa)	7.2 ± 0.7	7.2 ± 1.1	
PaO_2 (T0)			
(mmHg)	130 ± 102	136 ± 86	0.81
(kPa)	17.4 ± 13.6	18.1 ± 11.5	
PaO_2 (T30)			
(mmHg)	251 ± 94	269 ± 97	0.47
(kPa)	33.5 ± 12.6	35.8 ± 13.0	

$PaCO_2$, arterial carbon dioxide partial pressure; PaO_2, arterial oxygen partial pressure.

data and group allocation of the rabbits receiving either medetomidine-ketamine (MK) or medetomidine S(+)-ketamine (MS) intranasally. Body position during drug administration was different in the centres with sternal positioning (S) in Centre 1 and dorsal recumbency (D) in Centre 2.

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